

Determination of Bisphenol A and Bisphenol AF in Vinegar Samples by Two-Component Mixed Ionic Liquid Dispersive Liquid-Phase Microextraction Coupled with High Performance Liquid Chromatography

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Summary: This paper describes a sensitive and simple method for the detection of bisphenol A (BPA) and bisphenol AF (BPAF) in vinegar samples using two-component mixed ionic liquid dispersive liquid-liquid microextraction coupled with high performance liquid chromatography. In this work, BPA and BPAF were used as the target analytes, and two-component mixed ionic liquid included 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4Mim]PF_6$) and 1-hexyl-3-methyl-imidazolium hexafluorophosphate ($[C_6Mim]PF_6$) was used as the extraction solvent for the first time here. Parameters affecting the extraction efficiency were investigated. Under optimum conditions, better linear relation were discovered in the scope of 1.0-100 $\mu\text{g/L}$ for BPA and 2.0-150 $\mu\text{g/L}$ for BPAF, respectively. Detection limits of this method based on the signal-noise ratio (SNR=3) ranged from 0.15 to 0.38 $\mu\text{g/L}$. The efficiencies of proposed method have also been demonstrated with spiked real vinegar samples. The result show this method/procedure to be a more efficient approach for the determination of BPA and BPAF in real vinegar, presenting average recovery rate of 89.3-112 % and precision values of 0.9-13.5% (RSDs, n = 6). In comparison with traditional solid phase extraction procedures this method results in lower solvent consumption, low pollution levels, and faster sample preparation.

Keywords: Bisphenol A; Bisphenol AF; Vinegar; Ionic liquids; Dispersive liquid-phase microextraction.

Introduction

There has been a worldwide scientific and public discussion about the potential consequences of chronic dietary exposure to endocrine disruptors compounds (EDCs) during the last decade. Even at low concentrations, long-term exposure to EDCs is of toxicological concern and this increases when humans are exposed to mixtures of similar acting EDCs [1]. Both of bisphenol A (BPA) and bisphenol AF (BPAF) are EDCs. As major raw material of epoxy resin and carbonic ester plastic, they have been widely used in food packaging materials. They may migrate and transfer to drinks and food, and the potential risk to human and animal health has aroused more and more concern throughout the world [2]. More importantly, bisphenol A was found to possibly cause various kinds of cancer, pleiotropic damage in the brain and cardiovascular system [3]. In addition, BPAF may cause tissue disorganization and promote the proliferation and metastasis of cancer cells [4]. Several reports have investigated BPA levels in food [5], however, very few studies on the possible release of BPAF from plastic vinegar bottles have been reported. BPA has been demonstrated to be easily released from carbonic ester plastic and epoxy resin in acidic environments [6], and this may also occur with BPAF. In this paper the simultaneous determination of BPA and BPAF, and a precise, simple and easily operated method for general

measurement of BPA and BPAF levels from plastic vinegar bottles is described. Research was based on the fact that vinegar can contribute to a non-negligible amount in daily diet, due to its common use in China.

Low levels of BPA such as those reported can't be detected by conventional techniques [7] requiring the reduction of final extract volumes to attain higher concentration of target analytes. Some papers have been described the analytical method of these analytes, such as solid phase microextraction (SPME) [8] and solid phase extraction (SPE) which have shown good result [9]. Recently, a new type of microextraction, named ultrasound-assisted emulsification microextraction (USAEME) has made great strides. This technique results in a more efficient enrichment, lower solvent consumption, and faster sample preparation. It is quick, easy, inexpensive, simple, and compatible with many analytical equipment [10]. In this work, USAEME was used to analyze the amounts of BPA and BPAF in vinegar. High-density solvent was often used in USAEME as the extracting solvent, such as chlorobenzene, carbon tetrachloride, tetrachloroethylene etc [11]. These solvents would generate secondary pollution. Moreover it has been reported that ionic liquids (ILs) are excellent extractive agents compared with conventional

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solvents with the advantage of tunable viscosity, high thermal stability, miscibility with organic solvents and water [12]. Furthermore the physical and chemical properties of ILs can be modified by optimal anions and cations [13]. However, BPA and BPAF are different in physical properties therefore an extracting agent can't improve the efficiency of extraction. It is necessary to synthesize mixed-ILs to extract and then detect BPA and BPAF simultaneously. To our knowledge, no correlative literatures on appropriate ILs for the enrichment of BPAF and BPA simultaneous have been reported. In this paper, USAEME method was described for the detection of BPA and BPAF in vinegar using mixed-ILs coupled with HPLC-UV.

Results and Discussion

The extraction efficiency depends on some factors, such as pH, the concentration of extraction agent and ionic strength. In this paper, the type of ILs, and optimization and quality parameters of the USAEME method were investigated to get the best extraction efficiency.

Type of Ionic Liquid

In this paper, the most important question to consider was which extraction solvent was the most efficient. ILs has been used as green solvents and possible replacements for traditional solvents for a wide range of applications [14] as they are media resulting from combinations of various anions and organic cations. The extraction ability of ILs can be modified by optimal anion and cation. The structural variation of the hydrocarbyl groups in the ILs has the different influences on the extractability of various polar molecules. In this work, some ILs were synthetized from 3-methylindole by changing hydrocarbyl groups, the synthetic method was shown (Fig. 1). The extraction efficiency of various ILs was studied, and the results were shown in Fig. 2. From Fig. 2, it can be seen that peak area of BPA and BPAF reach the maximum when the number of hydrocarbyl groups of IL_S was 4 and 6 respectively. The results show that maximum amount of BPA and BPAF were transferred into the 1-butyl-3-methylimidazolium hexafluorophosphate (IL₁, number of hydrocarbyl groups is 4) and 1-hexyl-3-methyl-imidazolium hexafluorophosphate (IL₂, number of hydrocarbyl groups is 6) respectively. Therefore, 1-butyl-3-methylimidazolium hexafluorophosphate (IL₁) and 1-hexyl-3-methyl-imidazolium hexafluorophosphate (IL₂) were employed.

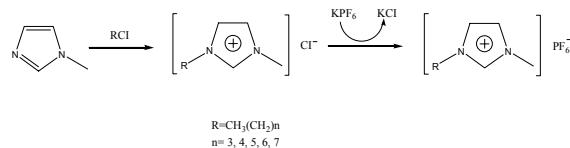


Fig. 1: Synthesis ionic of liquids.

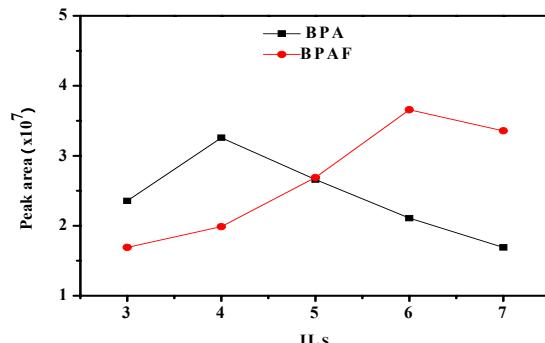


Fig. 2: Effect of ionic liquid. (Conditions; spiked concentration, 10 µg/L for BPA, 15 µg/L for BPAF 60 °C, 40kHz, 5 min ultrasonification time, ILs volume 35 µL and IL₁:IL₂ ratio of 2:4). X-axis data shows number of hydrocarbyl groups of ionic liquids while peak area is plotted in the y-axis.

The volume ratio of IL₁ and IL₂ was a critical factor, which would have a significant effect on extraction efficiency. Volume ratio was optimized within the range of 1:0-0:1(IL₁: IL₂; V/V), and the results were shown in Fig. 3. Best experimental results were obtained with the volume ratio of IL₁ and IL₂ at 2:4

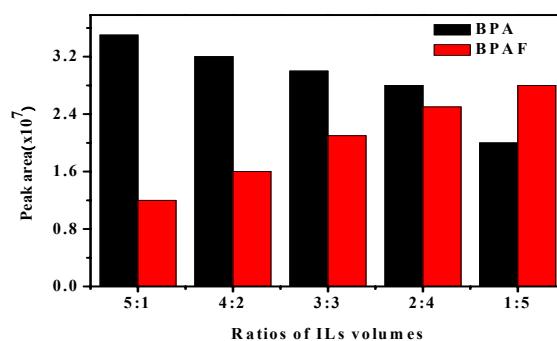


Fig. 3: Effect of ratios of ILs volumes. (Conditions; spiked concentration, 10 µg/L for BPA, 15 µg/L for BPAF 60 °C, 40kHz, 5 min ultrasonification time and ILs volume 35 µL). Ratios of mixed ILs volumes are plotted on the x axis while the peak area is plotted on the y-axis.

The volume of ILs (IL₁+IL₂) is another

important factor which can play an important role in the extraction process. Larger volume of ILs could result in the increase of volume of precipitation, which led to a lower enrichment factor. ILs volume was investigated within the range of 20-45 μL . The results are shown in Fig. 4. From these results, it could be seen that the peak areas of BPA and BPAF reached a maximum at 35 μL , and then decreased in the later phase gradually. Therefore, 35 μL of ILs was used in this paper.

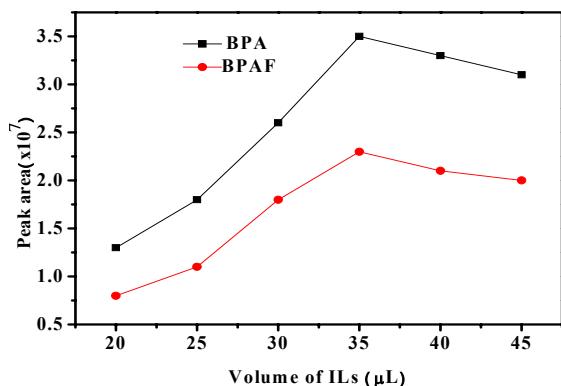


Fig. 4: Effect of ILs volume. (Conditions; spiked concentration, 10 $\mu\text{g/L}$ for BPA, 15 $\mu\text{g/L}$ for BPAF 60 $^{\circ}\text{C}$, 40kHz, 5 min ultrasonification time and IL₁:IL₂ ratio of 2:4).

Temperature is an important parameter, which governs ILs dispersion into the sample. Because the diffusion rate of the target analytes was very low at low temperature, so extraction efficiency was low. As temperatures rise, the mass transfer coefficients also increases. The rise of temperature not only enhances the transference into the ILs droplets but it also enhances migration out from the ILs. So, together, a reasonable temperature is very important. Temperature was examined within the scope of 40-80 $^{\circ}\text{C}$. Results were shown in Fig. 5. It was found that the migration out from the ILs droplets was larger than the transference into when temperatures exceeded 60 degrees. So a temperature of 60 $^{\circ}\text{C}$ was employed in all further analysis.

Optimization of the Method

It has been recently reported that the utilization of ultrasound increases the efficiencies in extraction process [15]. Under the ultrasound assistance conditions, a contact area enlargement region is formed between the aqueous phase and extraction solvent. Then, extraction solvent can disperse into the aqueous solution completely.

Ultrasonic frequency and ultrasonification time were also studied, and the results are shown in Fig. 6. From Fig. 6, it was found that the peak areas of the analytes increase with increasing the ultrasonic frequency up to 40 kHz after which it remained almost constant. Moreover the peak areas increase in the first five minutes after which it remain unchanged. Consequently, 5 min of ultrasonification time was chosen as the optimum time, 40 kHz of ultrasonic frequency was employed.

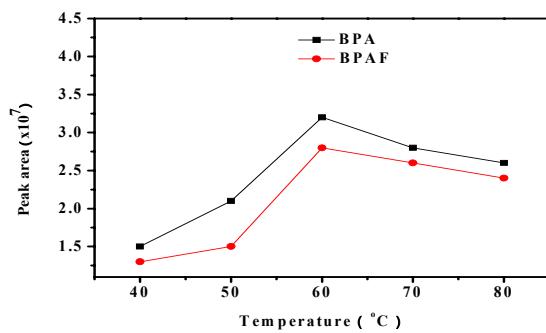


Fig. 5: Effect of temperature. (Conditions; spiked concentration, 10 $\mu\text{g/L}$ for BPA, 15 $\mu\text{g/L}$ for BPAF, 40kHz, 5 min ultrasonification time, ILs volume 35 μL and IL₁:IL₂ ratio of 2:4).

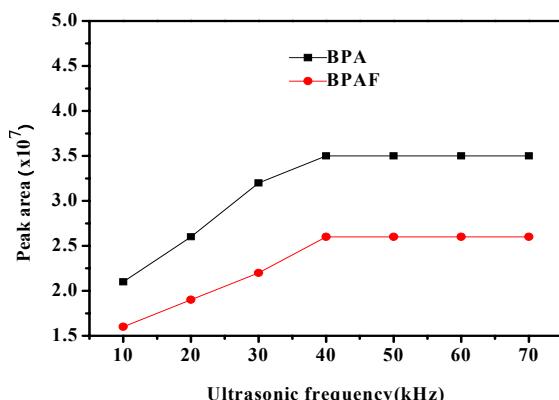


Fig. 6: Effect of ultrasonic frequency and ultrasonification time. (Spiked concentration, 10 $\mu\text{g/L}$ for BPA, 15 $\mu\text{g/L}$ for BPAF 60 $^{\circ}\text{C}$, ILs volume 35 μL and IL₁:IL₂ ratio of 2:4).

Analytical Performance of the Method

Precisions, detection limits and linear range were studied under optimum conditions (60 $^{\circ}\text{C}$, 40kHz, 5 min ultrasonification time, ILs volume 35 μL and IL₁:IL₂ ratio of 2:4). The results were listed in Table-1. Reproducibility experiment was investigated

by 6 individual experiments carried out with deionized water. The correlation coefficients ranged from 0.9991 to 0.9996. Good linear relation was discovered in the range of 1.0-100 µg/L for BPA and 2.0-150 µg/L for BPAF, respectively. Detection limits of this method based on the signal-noise ratio (SNR=3) ranged from 0.15 to 0.38 µg/L. The LODs were below the maximum allowable concentration of BPA and BPAF. This method could be used for detecting the residual concentration of BPA and BPAF in vinegar.

Table-1: Linear ranges and detection limits of BPA and BPAF by proposed method.

Analyte	Calibration range (µg/L)	R ²	Precision (RSD%, n= 6)	Detection limits (µg/L)
BPA	1-100	0.9991	3.7	0.15
BPAF	2-150	0.9996	4.2	0.38

In the meantime, a comparison was made to compare this method with solid phase extraction [16]. The results are given in Table-2. Although the proposed method show much less detection limits than SPE, the linear range is more widely than SPE. Furthermore, In comparison with SPE the proposed method results in lower solvent consumption, low pollution levels, and faster sample preparation. Therefore, these merits demonstrated that the proposed method would be a very effective technique and would possess certain practical value in the area of analysis field.

Table-2: Detection limits and linear ranges for the enrichment of BPAF and BPA by solid phase extraction.

Analyte	Calibration range (µg/L)	R ²	Precision (RSD%, n= 6)	Detection limits (µg/L)
BPA	0.5-50	0.9993	2.1	0.10
BPAF	0.5-50	0.9997	3.3	0.25

Real Vinegar Sample Analysis

Several brands of vinegar were analyzed with this method. The results were shown in Table-3. Results showed that the recoveries well ranged from 89.3 % to 112 % with the precisions of 0.9-13.5%. These results show that the method is a reliable method and will have better development prospect in the future.

Experimental

Instrumentation

Nuclear magnetic resonance spectra were recorded at a Bruker DRX-500 NMR spectrometer

using Me₄Si as an internal standard. The HPLC system consisted of four Agilent 1200 series LC-20AT pumps and SPD-M20A DAD detector (Agilent Technologies Inc., Santa Clara, USA). The chromatographic column was a 250mm×4.6mm column (C₁₈, particle size 5µm). The mobile phase consisted of acetonitrile and pure water (45/55, v/v). The mobile phase flow-rate was 1.0 mL/min, the injection volume was 20 µL, the column temperature was 25 °C and detection wavelength was 230 nm.

Material and Reagents

Vinegar of different brands (Lufeng (1), Baoning (2), Sanjia (3) and Laochen (4)) were purchased in August 2012 from Chinese supermarkets. All samples were sealed tightly and stored in a dry place at room temperature before analysis. Water used was purified with a purity water system (Chengdu, China). Acetonitrile (HPLC grade) was purchased from Fisher Chemicals (New Jersey, USA). Bisphenol A standard (\geq 99 %) was purchased from Sigma-Aldrich (U.K.) and Bisphenol AF standard (\geq 99 %) from Aladdin (ShangHai, China). BPA and BPAF standard solutions were prepared in methanol and stored at 4°C. 1-butyl-3-methylimidazolium hexafluorophosphate ([C₄Mim]PF₆, IL₁) and 1-hexyl-3-methylimidazolium hexafluorophosphate ([C₆Mim]PF₆, IL₂) were synthesized by our group as Fig. 1.

Method

Vinegar sample (10 mL) was placed in a 15 mL glass centrifuge tube. The pH value of sample was adjusted to 7 using ammonia solution. The solution was centrifuged for 5 min at 1500 rpm. The supernatant was transferred to a new tube and was spiked with a this standard solution [BPA(10 µg/L) and BPAF(15 µg/L)]. A mixture of 35 µL ionic liquids [[C₄Mim]PF₆(IL₁): [C₆Mim]PF₆(IL₂)=2:4; volume ratio] was injected into vial. The centrifuge tubes were heated in ultrasonic generator at 40 kHz with temperature of 60 degrees. The mixed ionic liquids (IL₁+IL₂) were dissolved completely. A cloudy solution was formed after the centrifuge tube was cooled with ice water for 10 min. The emulsion was disrupted by centrifuging for 5 min at 4000 rpm. After the upper liquid was removed, the low sediment was dissolved in 0.1 mL of acetonitrile and 20µL was injected for HPLC analysis.

Table-3: Recoveries and RSD of samples by this method.

Analyte	Spiked level ($\mu\text{g/L}$)	Lufeng®		Baoning®		Sanjia®		Laochen®	
		RR ^a	RSD (%)						
BPA	1	105	9.3	108	6.9	89.3	10.2	92.5	8.6
	100	98.3	0.9	101	2.5	102	1.5	99.3	2.8
BPAF	2	106	11.7	112	8.9	103	13.5	109	9.5
	150	101	2.1	97.5	0.9	97.8	3.1	103	1.7

^aAverage recovery rate for n=6.

The SPE method employed was as follows: the cartridge [Hydrophilic Lipophilic Balance (HLB), 0.5g] was washed by 10 mL of methyl alcohol and 10 mL of ultrapure water before extraction. The 10 mL of sample was percolated through the cartridge. Five mL of ultrapure water was used to remove impurities retained in the cartridge. Then, the cartridge was dried under vacuum for 10 mins, and the retained analytes were eluted with methanol. The eluate was dried under nitrogen. After this the residue was dissolved in 100 μL methanol and 20 μL of the sample solution was injected for HPLC analysis.

Conclusion

This paper describes two-component mixed ionic liquid dispersive liquid-liquid microextraction combined with high performance liquid chromatography for the detection of BPAF and BPA in vinegar. Final results on the monitoring of BPA and BPAF demonstrated good linearity, the low detection limits, the repeatability and sensitivity of this method would make it become a viable alternative and an effective method, with good prospect of application in the future.

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